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SELECTION FOR OLEIC, LINOLEIC AND LINOLENIC ACIDS IN SEED OIL OF RAPESEED (*BRASSICA NAPUS* L.)

by

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ABSTRACT

The oleic, linoleic and linolenic acid content of rapeseed (Brassica napus L.) oil was investigated in F_2, F_3 and F_4 populations of three crosses. The populations were derived from three strains of rapeseed that produce seed oil practically free of eicosenoic and erucic acids and differing in oleic, linoleic and linolenic acid content. Parent 1 (P_1) was high in oleic and low in linoleic acid. Parent 2 (P_2) was intermediate in value for both fatty acids and parent 3 (P_3) was characterized as being low in oleic and high in linoleic acid content. Selections of F_2 single plants representing the ten highest single plant values, the ten lowest and ten intermediate values were made for each of the three C-18 fatty acids in each cross. Seed from the field grown F_2 single plant selections was advanced through the F_3 generation in the greenhouse and the F_4 generation in the field. Bulk F_2, F_3 and F_4 seed samples were analysed for fatty acid composition.

Intergeneration simple correlation and Spearmen's rank correlation values were highly significant for oleic and linoleic acids in $P_1 \times P_2$ and $P_1 \times P_3$ but not in $P_2 \times P_3$. The intergeneration correlations for linolenic acid were almost all non-significant in all three crosses. Regression heritabilities and heritabilities from analysis of variance indicated high heritabilities for oleic and linoleic acids but low heritabilities for linolenic acid. Heritabilities were generally higher in

$P_1 \times P_2$ and $P_1 \times P_3$ than in $P_2 \times P_3$. Significant differences observed between the low and high F_2 class means were also observed for the F_3 and F_4 progeny means of these classes for oleic and linoleic acids in $P_1 \times P_2$ and $P_1 \times P_3$ but not in $P_2 \times P_3$. In general, these differences were not observed for linolenic acid in all three crosses. Average coefficients of variation indicated that selection in the F_2 was successful in assigning a greater number of homozygous plants to the low and high classes than to the intermediate class on the basis of oleic and linoleic acid content. However, this was not the case for linolenic acid.

Correlations between oleic versus linoleic and oleic versus linolenic were negative and highly significant. Correlations between linoleic versus linolenic were positive, significant in all but two populations and lower in magnitude than oleic versus linoleic and oleic versus linolenic. The linolenic/linoleic ratio was an unsatisfactory indicator of linolenic acid content in F_3 and F_4 families.

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

The center of origin of rapeseed is considered to be Asia (Downey 1966). Brassica napus L. evolved as an amphidiploid from a cross of Brassica campestris L. and Brassica oleracea L. Oilseed rapes were reported in India as early as 2000 B.C. By the sixteenth century, B. napus and B. campestris were under cultivation in Belgium, Holland and Germany where pressed seed oil had traditionally been used for illumination and soap making.

By the nineteenth century rapeseed was cultivated throughout Europe and was first cultivated in North and South America prior to and during World War II. B. napus was introduced into Canada from Argentina and first grown commercially in 1942 (Downey and Bolton 1961). B. campestris was subsequently introduced. Production of rapeseed in Canada is centered in the prairie provinces. The greatest production was achieved in 1971 from 5.3 million acres. Approximately 75% of the acreage is B. campestris which is cross-pollinated and early maturing but has less yield potential than B. napus which is largely self-pollinated and late maturing.

The chief economic value of rapeseed oil is in salad and cooking oils, margarine and shortening. In world production of edible oil crops, rapeseed ranks fourth after soybeans, sunflower and groundnut.

Increased demand for rapeseed oil is expected to result from improvements in oil quality which is largely determined by its

fatty acid composition. The major fatty acid constituents of traditional rapeseed oils are palmitic, oleic, linoleic, linolenic, eicosenoic are erucic acids. Nutritional studies have indicated that eicosenoic and erucic acids are detrimental to the health of monogastrics (Beare-Rogers 1970). A major nutritional improvement in oil quality has been achieved as a result of breeding low eicosenoic and low erucic acid cultivars. The fatty acids oleic, linoleic and linolenic constitute approximately 95% of the fatty acids in low erucic acid cultivars. Further improvements in oil quality require the incorporation of low linolenic and high linoleic characters into existing low erucic cultivars. Removal of linolenic acid is expected to reduce processing costs and improve the flavour stability of the oil (Downey 1966). An increase in linoleic acid content will improve the nutritional value of the oil.

Success in breeding low linolenic and high linoleic cultivars depends on the ability of the plant breeder to make selections for desirable levels of these two fatty acids. The present study was undertaken to evaluate early generation selection for different levels of oleic, linoleic and linolenic acids.

LITERATURE REVIEW

Genetic variability for fatty acid composition of seed oil of rapeseed was first investigated on a cultivar basis. High erucic acids cultivars (four Brassica napus L. and three Brassica campestris L.) were grown at seven stations in western Canada and analyzed for fatty acid composition (Craig and Wetter 1959). There was significant variation among cultivars for the major fatty acids including: oleic (18:1), linoleic (18:2), linolenic (18:3), palmitic (16:0), eicosenoic (20:1) and erucic (22:1). However, the greatest variation occurred among oleic, linoleic and erucic acids. In a similar study six cultivars (three B. napus and three B. campestris) were grown at twenty-two stations in western Canada (Craig 1961). Significant variation occurred among stations for all fatty acids and among cultivars for each fatty acid except linolenic. In both studies, the differences among cultivars were primarily due to the difference in fatty acid composition between the two species.

Low erucic acid strains of B. napus (Stefansson et al 1961) and B. campestris (Downey 1964) were isolated as a result of inbreeding and selection for low erucic acid content. Selection for low erucic acid strains resulted in a simultaneous selection for low eicosenoic acid content. There was a corresponding increase in oleic and linoleic acid content (Downey and Craig 1964).

Data from self-pollinated and reciprocally cross-pollinated seed of low and high erucic acid plants demonstrated that the level of erucic acid in B. napus was controlled by the embryo genotype rather than the maternal plant genotype (Downey and Harvey 1963). Oleic acid was also controlled by the embryo genotype in seeds that are segregating for erucic acid content because of the large negative correlation (-.98) between percent oleic and percent erucic acid (Downey and Craig 1964). Erucic acid levels in a single cross of B. napus were controlled by a two-gene system with equal and additive effects (Harvey and Downey 1964). Each allele for high erucic acid contributed 9 - 10% to the total erucic acid content. There is evidence that the same two-gene-system controlled eicosenoic acid content but that high values of eicosenoic acid were dominant to low values (Kondra and Stefansson 1965). Erucic acid content of B. campestris was controlled by the embryo genotype and conditioned by a single gene system acting in an additive manner (Dorrell and Downey 1964). Each gene contributed 10 - 12% to the total erucic acid content.

The biosynthetic pathway for eicosenoic and erucic acids consists of carbon chain elongation by the addition of acetate molecules to the carboxyl end of oleic acid (Downey and Craig 1964). A separate biosynthetic pathway for linoleic and linolenic acids consists of desaturation with oleic acid as a precursor (Downey 1966). Low erucic acid strains were selected as a result of the presence of a genetic block between oleic and eicosenoic acids in

the elongation pathway. Much less success has been achieved in altering the desaturation pathway of the C-18 fatty acids. Three cycles of selection for low linolenic acid content produced limited genetic advance in the high erucic varieties Nugget and Golden (Downey 1965). The greater success was achieved with Golden where linolenic content was only reduced from 6.80% to 6.20%. Use of the chemical mutagen, ethylmethylsulfonate, in the low erucic variety Oro indicated that it is possible to select progeny having large differences in linolenic acid content (Rakow and McGregor 1973). One stable mutant had 5% and a second stable mutant had 20% linolenic acid. Both mutants had levels of 16 - 20% linoleic acid in the seed oil. However, many selections were unstable, reverting back to the original Oro fatty acid composition after two or three generations of self-pollination. Selection was performed by using the ratio of linolenic to linoleic acid (18:3/13:2 ratio) as determined by a photometric method on segregating M2 half-seeds from M1 plants.

The embryo is the major source of fatty acid synthesis in the seed and constitutes approximately 85% of the mature seed weight. A single cell layer of endosperm and a thin seed coat (testa) contribute relatively little to the total seed oil. Therefore, it is expected that the embryo genotype would control the fatty acid composition of seed oil. However, there is evidence that the maternal plant genotype may exercise some control over C-18 fatty acid synthesis in low erucic B. napus lines (Kondra and Stefansson 1970, Thomas and Kondra 1973).

Thomas (1974) investigated the inheritance of C-18 fatty acids in self- and cross-pollinated seed on parental lines and reciprocal F_1 and F_2 populations derived from three crosses produced from three low erucic B. napus lines. Cross-pollinated seed in two of the three crosses showed a maternal genotype effect on oleic and linoleic acid content. The third cross indicated embryo control for these two fatty acids. Embryo and maternal genotype control was found for linolenic acid. No cytoplasmic effects were evident in the F_1 and F_2 generations. Data from F_1 population means indicated an additive gene system for oleic and linoleic acids in one of the three crosses while the other two crosses indicated partial dominance for high oleic values and low linoleic values. Complete dominance for low linolenic acid content was observed. Broad sense heritability estimates and number of effective factors were calculated by three methods. The heritabilities and number of effective factors calculated by the different methods produced essentially the same results within each cross. Average heritabilities within each cross for oleic acid (75%, 77% 58%) and linoleic acid (81%, 81%, 43%) were relatively high, while those for linolenic acid (54%, 41%, 30%) were generally lower. The average number of effective factors within each cross varied from cross to cross for each fatty acid. The average number of effective factors in the three crosses were 2, 6 and 3 for oleic acid and 3, 5 and 4 for linoleic acid. The average number of effective factors for linolenic acid were 0, 2 and 3. It was postulated that one genetic system controlled both oleic and linoleic acid content (Thomas 1974).

Inheritance of fatty acid composition has been studied in other oilseed crops. A single locus controlling oleic and linoleic acid content in corn was reported with low linoleic acid content dominant to high linoleic acid and high oleic acid content dominant to low oleic acid content (Poneleit and Alexander 1965). The major fatty acids in corn demonstrated several types of gene action for each fatty acid in four different crosses (Jellum 1966). Additive, dominant and heterotic gene effects were observed. Fatty acid analysis of self- and cross-pollinated seed on the parental corn lines indicated significant maternal effects in some crosses. Another study with corn found largely additive gene action for oleic and linoleic acids and significant cytoplasmic effects for these two fatty acids (Poneleit and Bauman 1970). Further studies with linoleic acid in three corn crosses indicated the presence of two loci in one cross, cytoplasmic effects in another cross and variable levels of dominance in all three crosses (de la Roche et al 1971). A single locus with partial dominance for low linoleic acid along with another independently acting locus were found to control levels of linoleic acid in corn (Poneleit 1972). There is evidence for complete embryo control of oleic and linoleic acid content in safflower (Knowles and Hill 1964). Iodine value of safflower oil was reported to be determined largely by the embryo genotype and be highly heritable (Yermanos et al 1967). The data also indicated that one locus controlled levels of oleic and linoleic acids with partial dominance for high linoleic and low oleic values. Stearic

acid in safflower seed oil was controlled by two alleles at one locus with partial dominance for low levels (Ladd and Knowles 1970). The levels of oleic, linoleic and linolenic acids in flax were found to be largely under the control of the embryo genotype (Yermanos and Knowles 1962). Partial dominance for low linolenic acid content and high oleic acid content was observed in flax (Comstock et al 1960). High heritabilities were calculated for stearic (93.1%), oleic (65.0%) and linolenic (72.9%) in field grown generations but lower heritabilities for these fatty acids (1.3%, 3.7% and 4.7% respectively) were calculated for generations grown in growth chambers. Levels of C-18 fatty acids in soybeans were primarily under maternal control (Brim et al 1968). It was found that levels of linoleic and linolenic acid in soybeans were quantitatively inherited with some transgressive segregation to low values (White et al 1961). The fatty acid values were highly influenced by the environment.

Environmental effects on fatty acid composition of rapeseed have been studied. It was observed that the fatty acid composition of high erucic rapeseed showed a more complex response over a temperature range varying from 10°C. to 26.5°C. than did sunflower, flax, safflower or castor bean (Canvin 1965). At 10°C. and 16°C. erucic acid levels were higher and oleic levels lower than at 21°C. and 26.5°C. Delayed seeding of high erucic acid cultivars of B. napus and B. campestris resulted in decreased oleic, and increased linoleic and linolenic acid content in relation to early seeded

material (Gross and Stefansson 1966).

The percent composition of C-18 fatty acids was found to become fixed between 21 and 28 days after pollination (DAP) or approximately half way through seed development of a low erucic acid strain of B. napus (Fowler and Downey 1970). It was suggested that variation in fatty acid composition of the low erucic strain during early stages of seed development was due to the changing contributions of the testa, nucleate endosperm and embryo. A high erucic acid cultivar, Nugget, showed a rapid increase in oleic acid over the period 21 to 28 DAP. Percent erucic acid accumulated rapidly from 21 DAP until seed maturity at the expense of oleic acid content. Hence, relative C-18 fatty acid composition of the high erucic cultivar was found to vary throughout seed development.

Two low erucic acid B. napus strains were used to derive parental and backcross populations from which the following ranges of correlation coefficients were calculated for pairs of C-18 fatty acids: oleic and linoleic -.85 to -.98, oleic and linolenic -.58 to 0.70, linoleic and linolenic .31 to .55 (Stefansson and Storgaard 1969). These correlation coefficients were calculated from fatty acid values expressed as a percent of total fatty acids. When the fatty acid values were expressed as percent of seed weight, the above correlations were reduced in magnitude. Expressing fatty acids as percent of seed weight means that any variation in fatty acid composition of the seed oil can be accompanied by variation in other seed components such as the protein and carbohydrate fractions.

Therefore, the chance of obtaining large negative correlations between pairs of fatty acids is reduced. The ranges of correlation coefficients for percent fatty acids, calculated as percent of total fatty acids, of parental, F_1 and F_2 populations derived from three low erucic B. napus lines were: oleic and linoleic -.68 to -.96, oleic and linolenic -.57 to -.75, linoleic and linolenic -.31 to .55 (Thomas 1974).

Correlation coefficients for pairs of C-18 fatty acids in other oil seed crops indicate strong associations for some fatty acids. A correlation coefficient of 0.99 was reported for oleic and linoleic acids in safflower (Yermanos et al 1967) and -.97 in corn (Poneleit and Bauman 1970). Correlations between oleic and linolenic in flax ranged from -.77 to -.97 (Comstock et al 1960). Correlations between linoleic and linolenic in soybeans were consistently high (.74 and .76) in one study (Howell and Collins 1957) but ranged from .13 to .96 in another study (White et al 1961).

MATERIALS AND METHODS

Plant Materials

Three strains of rapeseed (Brassica napus L.) having very low levels of eicosenoic and erucic acids in their seed oil but differing in oleic, linoleic and linolenic acid content were used as parental lines. Each line originated from a single plant selection from the cultivar Liho and inbred for 8 generations. Self-pollinated seed from these parental lines produced seed oil with the fatty acid composition given in table 1 (Thomas 1974).

Table 1

Fatty acid composition of oil from self-pollinated seed of three strains of rapeseed

Parent designation	Fatty acids as percent of total fatty acids					
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
1	4.7	69.7	12.8	12.2	0.7	t*
2	4.9	54.2	27.4	12.0	1.7	t
3	6.0	42.6	35.6	15.2	0.5	t

*t = trace

Plants from the three strains were combined in a 3 x 3 diallel cross. The F_1 plants from the diallel cross were self-pollinated in the greenhouse to produce F_2 seed. The F_2 populations were grown in the field during the summer of 1971. Field grown seed

from F_2 single plants in each of the three crosses, $P_1 \times P_2$, $P_1 \times P_3$, and $P_2 \times P_3$ was analyzed for fatty acid composition. The total number of plants in the F_2 of each population was 105 in $P_1 \times P_2$, 96 in $P_1 \times P_3$ and 99 in $P_2 \times P_3$. The parental, F_1 and F_2 populations were developed and investigated by P. M. Thomas (1974).

Material for the present study was selected on the basis of C-18 fatty acid composition of seed of field grown F_2 plants of each cross. Ten single plant selections were made at each of three levels for each C-18 fatty acid within each cross. For each fatty acid the three levels were: the ten highest single plant values, the ten lowest and ten intermediate values. The potential number of F_2 plants selected was 270. However, some F_2 plants selected for a given fatty acid class within a cross also corresponded to a selected class for another fatty acid. Consequently, the total number of F_2 plants selected was 169. Six seeds were randomly selected from each of these F_2 plants and planted in the greenhouse during the winter of 1972-73. In the spring of 1973, four F_3 plants in each family were individually harvested and threshed. Five seeds were taken from each of the four plants and bulked for fatty acid analysis to provide F_3 family mean values.

Remaining seed from the F_3 plants was planted in the field to produce four F_4 progeny rows from each F_3 family. The field plots were planted in a four replicate design with one F_4 progeny row from each F_3 family per replicate. Progeny rows were planted at a density of 1.2 grams of seed per 6 meter row with 30 centimeters between rows.

Due to a wet cool cummer, many plants were either killed by flooding or were immature. Therefore, the F_4 was harvested as a completely randomized trial with ten mature plants per family being selected at random. These plants were then individually threshed and F_4 family samples were obtained by bulking 1.5 gram samples from each of the ten plants per family. This seed was then ground in a blender and two sub-samples from each family were analyzed for fatty acid composition. The sub-sample values were averaged to provide F_4 family values. For each fatty acid in F_4 families of each cross, the three highest F_4 families, the three lowest F_4 families and three intermediate F_4 families were identified. Ten plants within each family were individually analyzed for fatty acid composition.

Fatty Acid Analysis

The fatty acid composition was determined by gas-liquid-phase chromatography. The methylesters for gas chromatography were prepared according to the method of Downey and Craig (1964).

Equipment:

1) Hewlett Packard 5750 Research Chromatograph equipped with a hydrogen flame ionization detector and two columns consisting of a 1/8" x 8' stainless steel tube containing Chromosorb G in the solid phase and 4% by weight butanediolsuccinate liquid phase.

2) Hewlett Packard 3370 B Integrator used to determine the areas of the gas chromatograph peaks in order to calculate fatty acids as percent of total fatty acid.

The following fatty acids were calculated after the gas chromatograph had been calibrated with standard samples: palmitic (16:0), oleic (18:1), linoleic (18:2), linolenic (18:3), eicosenoic (20:1), and erucic (22:1).

Statistical Procedures

For each fatty acid within each of the three crosses, simple correlation, Spearman rank correlation and simple regression were calculated (Steel and Torrie 1960) for F_3 family means with F_2 single plant values, F_4 family means with F_3 family means and F_4 family means with F_2 single plant values. Intergeneration regression coefficients were used as heritability estimates (Hanson 1963).

Analysis of variance for each fatty acid within each cross was performed for the three generations to provide broad sense heritability (h^2) estimates. In the model, single F_2 plants and their derived F_3 and F_4 families were considered as fixed effects while environments were random effects.

Model:

$$X_{ij} = \mu + F_i + E_j + I_{ij}$$

where X_{ij} is the single plant or family value in a given environment, μ is the population mean, F_i is the family effect, E_j is the environmental effect and I_{ij} is the interaction effect of the i^{th} single plant or family with the j^{th} environment.

The following analysis of variance table was used:

Source	d.f.	M.S.	E.M.S.
Families	f-1	M.S.F.	$\sigma^2 + e\sigma f^2$
Environments	e-1	M.S.E.	$\sigma^2 + f\sigma e^2$
Interaction	(f-1)(e-1)	M.S.I.	σ^2

From the analysis of variance performed on each of the three C-18 fatty acids in each cross, broad sense heritability (h^2) for each fatty acid was estimated as follows:

$$\sigma^2 e = \frac{\text{M.S.E.} - \text{M.S.I.}}{f} \qquad \sigma^2 f = \frac{\text{M.S.F.} - \text{M.S.I.}}{e}$$

$$h^2 = \frac{\sigma^2 f}{\sigma^2 + \sigma^2 f + \sigma^2 e}$$

A 3 x 3 factorial analysis of variance was performed on each fatty acid within each cross for the three classes of F_2 selected plants and their F_3 and F_4 progeny. For each fatty acid within each cross, differences among the three class means within each generation were tested by the method of least significant difference as outlined in Steel and Torrie (1960).

Coefficients of variation were calculated for the three high, intermediate and low value F_4 families for each fatty acid within each cross. The three coefficients of variation within each of the three sets of families were averaged.

Linolenic - Linoleic Ratio

Rakow and McGregor (1973) used the ratio of percent linolenic to percent linoleic acid (18:3/18:2) as a method of selecting mutants having varying levels of linoleic and linolenic acids. The range of those ratios having low levels of linolenic acid was .22 to .35 and the range of those ratios having high levels of linolenic acid was .47 to .88. The ratios were calculated in generations grown

under different environmental conditions.

In the present study, the ratio of linolenic to linoleic acid, based on bulk family values in the F_3 and F_4, was calculated for each cross. The ten lowest ratios representing approximately 20% of the population within each cross were identified. The ten families having the lowest levels of linolenic acid within each cross were also identified. The discrepency between these two methods of selection was tabulated as a means of determining the ability of the 18:3/18:2 ratio to identify low linolenic families.

RESULTS

Oleic Acid

a) Intergeneration statistics

For the cross $P_1 \times P_2$, the simple correlation values for F_3 versus F_2, F_4 versus F_3 and F_4 versus F_2 were highly significant (.76, .63 and .70 respectively) (Table 2). They were not significantly different from each other at the 5% level. The corresponding Spearman's rank values were highly significant and similar in magnitude (.70, .66 and .71). They were not significantly different from each other. The regression values were also highly significant (.58, .66 and .56) and were not significantly different from each other.

The three intergeneration simple correlation coefficients for $P_1 \times P_3$ were highly significant (.89, .74 and .72). The intergeneration Spearman's rank correlation coefficients were highly significant (.83, .70 and .58) as were the regression coefficients (.76, .58 and .48). There were no significant differences among the simple correlation coefficients. For Spearman's rank correlation and regression, the F_3 versus F_2 values were significantly higher than the F_4 versus F_2 values. The F_4 versus F_3 values were intermediate in magnitude and were not significantly different from the F_3 versus F_2 and F_4 versus F_2 values at the 5% level.

Table 2. Intergeneration simple correlation, Spearman rank correlation and regression coefficients for oleic acid values among F_2 single plant selections and their F_3 and F_4 progenies.

	Cross								
	$P_1 \times P_2$ (n=25)			$P_1 \times P_3$ (n=28)			$P_2 \times P_3$ (n=26)		
		rank			rank			rank	
Generations	r	r	b	r	r	b	r	r	b
$F_3 - F_2$	.76**a†	.70**a	.58**a	.89**a	.83**a	.76**a	.08a	.02a	.06a
$F_4 - F_3$	.63**a	.66**a	.66**a	.74**a	.70**ab	.58**ab	.32a	.31a	.25a
$F_4 - F_2$	.70**a	.71**a	.56**a	.72**a	.58**b	.48**b	.14a	.16a	.09a

** significant at 1% level

* significant at 5% level

† coefficients for each type of intergeneration statistic within each cross followed by the same letter are not significantly different at the 5% level.

For the cross $P_2 \times P_3$, the intergeneration correlations and regressions were non-significant. There were no significant differences among correlations or regressions. The three intergeneration simple correlation coefficients were .08, .32 and .14. The Spearman's rank correlation coefficients were .02, .31 and .16 and the regression coefficients were .06, .25 and .09.

Analysis of variance for cross $P_1 \times P_2$ for oleic acid content of F_2 single plant selections and their F_3 and F_4 progenies grown in three environments gave highly significant F values for environments and families (88.71 and 4.81 respectively) (Table 3). The heritability estimate for oleic acid content based on the analysis of variance was 31.8%. For $P_1 \times P_3$, the F values for environments and families were also highly significant (87.09 and 6.21 respectively). The heritability estimate was 39.0%. Similarly, the F values for $P_2 \times P_3$ were highly significant for environments and families (59.77 and 2.11 respectively). However, the heritability estimate was only 14.6%.

b) Means, ranges and standard deviations of F_2, F_3 and F_4 populations.

The cross with the highest mean oleic value in each generation was $P_1 \times P_2$ with means of 64.2%, 70.4% and 61.2% in the F_2, F_3 and F_4 respectively (Table 4). All three generation means differed significantly from each other at the 5% level. In cross $P_1 \times P_3$, the mean of the F_3 (65.9%) was significantly higher than the F_2 mean

Table 3. Analysis of variance and heritability estimates for oleic acid.

Cross	Source	d.f.	Mean square	F value	Heritability Estimate
$P_1 \times P_2$	Families	50	46.44	4.81**	31.8%
	Environments	2	856.94	88.71**	
	Interaction	100	9.66		
$P_1 \times P_3$	Families	49	64.50	6.21**	39.0%
	Environments	2	904.03	87.09**	
	Interaction	98	10.38		
$P_2 \times P_3$	Families	49	19.60	2.11**	14.6%
	Environments	2	554.06	59.77**	
	Interaction	98	9.27		

** significant at 1% level

* significant at 5% level

Table 4. Frequency distributions for oleic acid content of F_2 single plant selections and their F_3 and F_4 progenies.

Population	Class centers in percent of oleic acid																n	Mean	Standard Deviation
	46	48	50	52	54	57	58	60	62	64	66	68	70	72	74	76			
$P_1 \times P_2$																			
F_2			1	0	1	1	5	2	2	2	2	2	5	4	1		28	64.2a*	6.5
F_3								1	2	1	2	2	5	1	7	4	25	70.4b	5.0
F_4				1	3	3	3	6	0	2	7	1	1	1			28	61.2c	5.1
$P_1 \times P_3$																			
F_2	1	0	1	6	3	2	2	2	2	0	0	5	2	2			28	59.4a	7.8
F_3					1	1	3	2	4	3	2	2	1	2	5	2	28	65.9b	6.6
F_4				3	3	3	5	3	1	3	6	1					28	59.9a	5.2
$P_2 \times P_3$																			
F_2	1	3	5	2	4	2	4	6									27	54.0a	4.6
F_3			1	2	1	4	7	4	4	2	1						26	58.4b	3.8
F_4	1	0	3	7	8	4	3	1									27	53.8a	3.3

* generation means within each cross followed by the same letter are not significantly different at the 5% level (L.S.D.).

(59.4%) and the F_4 mean (59.9%). The F_2 and F_4 means were not significantly different from each other. The means of $P_2 \times P_3$ were lower in magnitude than the means of the other two crosses. The F_3 mean (58.4%) was significantly higher than the F_2 mean (54.0%) and the F_4 mean (53.8%). The F_2 and F_4 means were not significantly different from each other.

For $P_1 \times P_2$, the ranges of oleic acid content of the F_2 single plants, F_3 families and F_4 families were: 50.9% - 73.7%, 59.8% - 76.9% and 52.3% - 71.3% respectively. For $P_1 \times P_3$, the ranges of the F_2, F_3 and F_4 were: 45.8% - 71.6%, 53.5% - 76.2% and 51.1% - 68.3%. The ranges of the three generations in $P_2 \times P_3$ were: 45.1% - 60.7%, 50.7% - 65.0% and 45.5% - 61.7%.

The cross $P_1 \times P_3$ had the greatest variation for percent oleic acid of all three crosses with standard deviations of 7.8, 6.6 and 5.2 in the F_2, F_3 and F_4 respectively. Cross $P_1 \times P_2$ had standard deviations of 6.5, 5.0 and 5.1 for the three generations while $P_2 \times P_3$ had less variation with standard deviations of 4.6, 3.8 and 3.3 in the F_2, F_3 and F_4 respectively.

c) Means of selected F_2 classes and their F_3 and F_4 progenies.

For cross $P_1 \times P_2$, the low, intermediate and high F_2 class means were significantly different from each other at the 5% level (56.2%, 63.7% and 71.7% respectively) (Table 5). The mean of F_3 progeny of the F_2 intermediate class (71.4%) was significantly

Table 5. Means of oleic acid content for selected low, intermediate and high F_2 classes and their F_3 and F_4 progenies.

Cross	Class	Generation		
		F_2	F_3	F_4
$P_1 \times P_2$	Low	56.2 a* (n=8)	64.8 a (n=7)	58.0 a (n=8)
	Intermediate	63.7 b (n=10)	71.4 b (n=10)	61.4ab (n=10)
	High	71.7 c (n=10)	73.9 b (n=8)	63.7 b (n=10)
$P_1 \times P_3$	Low	51.8 a (n=10)	59.8 a (n=10)	57.3 a (n=10)
	Intermediate	57.8 b (n=9)	65.2 b (n=9)	57.5 a (n=9)
	High	69.4 c (n=9)	73.3 c (n=9)	65.2 b (n=9)
$P_2 \times P_3$	Low	48.5 a (n=9)	58.3 a (n=9)	53.8 a (n=9)
	Intermediate	53.8 b (n=8)	58.5 a (n=7)	53.4 a (n=8)
	High	58.9 c (n=10)	58.4 a (n=10)	54.1 a (n=10)

* class means within each generation within each cross followed by the same letter are not significantly different at the 5% level (L.S.D.).

higher than the mean of F_3 progeny of the F_2 low class (64.8%) but not significantly lower than the F_3 progeny mean of the F_2 high class (73.9%). The mean of F_4 progeny of the F_2 low and high classes (58.0% and 63.7% respectively) were significantly different from each other. The F_4 progeny mean of the F_2 intermediate class (61.4%) was not significantly different from either the low or high class progeny means.

For cross $P_1 \times P_3$, significant differences were observed among all F_2 class means (51.8%, 57.8% and 69.4%) and among the means of corresponding F_3 progenies (59.8%, 65.2% and 73.3%). However, there was no significant difference between the F_4 progeny mean of the F_2 low class (57.3%) and the F_4 progeny mean of the F_2 intermediate class (57.5%) which was significantly lower than the mean of the high class progeny (65.2%).

For $P_2 \times P_3$, significant differences were observed among the low, intermediate and high F_2 classes (48.5%, 53.8% and 58.9% respectively). No significant differences existed among the corresponding F_3 progenies (58.3%, 58.5% and 58.4%) or among the corresponding F_4 progenies (53.8%, 53.4% and 54.1%).

d) Average coefficients of variation for selected F_4 families.

On the basis of oleic acid content in F_4 families, the three highest value F_4 families, the three lowest value F_4 families and three intermediate value F_4 families were identified. Coefficients of variation were calculated for each family based on ten F_4 single

Table 6. Average coefficients of variation for oleic acid content for three families in each of the low, intermediate and high classes of the F_4 generation.

Cross	Class		
	Low	Intermediate	High
$P_1 \times P_2$	5.6% (n=27)	9.9% (n=17)	4.4% (n=25)
$P_1 \times P_3$	4.0% (n=22)	10.2% (n=30)	3.8% (n=26)
$P_2 \times P_3$	6.0% (n=24)	5.2% (n=25)	7.2% (n=25)

plant values. These coefficients were averaged to produce an average coefficient of variation (A.C.V.) corresponding to each of the three sets of families. For cross $P_1 \times P_2$, the A.C.V. values for the three low and three high families (5.6% and 4.4% respectively) were lower than the A.C.V. value for the three intermediate families (9.9%) (Table 6). The A.C.V. values for the three low (4.0%) and three high (3.8%) families of $P_1 \times P_3$ were also less than the A.C.V. value for the three intermediate families (10.2%). However, in $P_2 \times P_3$, the A.C.V. values for the three low and three high families (6.0% and 7.2% respectively) were slightly greater than the A.C.V. value for the three intermediate families (5.2%). Thus, in two crosses, there was a greater A.C.V. value for the intermediate families than for the low and high families. For the third cross, the three sets of families showed practically no difference among the A.C.V. values.

Linoleic Acid

a) Intergeneration statistics

For the cross $P_1 \times P_2$, the intergeneration simple correlation coefficients for F_3 versus F_2, F_4 versus F_3 and F_4 versus F_2 were highly significant (.86, .70 and .80 respectively) (Table 7). The corresponding Spearman's rank values were highly significant and of the same magnitude (.81. .68 and .78) as the simple correlation values. Intergeneration regression values were also highly

significant (.65, .60 and .52). No significant differences were observed among the intergeneration simple correlation, Spearman's rank correlation or regression values at the 5% level.

For the cross $P_1 \times P_3$, all of the intergeneration values were highly significant. The three intergeneration correlation coefficients were .92, .80 and .84 and the Spearman's rank correlation coefficients were .87, .78 and .80. The intergeneration regression coefficients were lower in magnitude (.75, .62 and .54). There were no significant differences among the simple correlation coefficients. There were no significant differences among rank correlation coefficients. The F_3 versus F_2 regression coefficient was significantly higher than the F_4 versus F_2 regression coefficient. The F_4 versus F_3 value was not significantly different from the F_3 versus F_2 and F_4 versus F_2 values.

Intergeneration values in the cross $P_2 \times P_3$ were variable in magnitude. The intergeneration simple correlation values were .36, .02 and .53. The Spearman's rank correlation values were .32, .03 and .55 and the regression values were .24, .01 and .29. Only the F_4 versus F_2 intergeneration values were significant while the F_3 versus F_2 and the F_4 versus F_3 values were non-significant. Simple correlation and Spearman's rank correlation for the F_4 versus F_3 were significantly lower than the F_4 versus F_2 values. The F_3 versus F_2 values were intermediate and not significantly different from the F_4 versus F_3 and F_4 versus F_2 values. There were no significant differences among regression values.

Table 7. Intergeneration simple correlation, Spearman rank correlation and regression coefficients for linoleic acid values among F_2 single plant selections and their F_3 and F_4 progenies.

	Cross								
	$P_1 \times P_2$ (n=27)			$P_1 \times P_3$ (n=29)			$P_2 \times P_3$ (n=25)		
		rank			rank			rank	
Generations	r	r	b	r	r	b	r	r	b
$F_3 - F_2$	.86**a†	.81**a	.65**a	.92**a	.87**a	.75**a	.36ab	.32ab	.24a
$F_4 - F_3$	.70**a	.68**a	.60**a	.80**a	.78**a	.62**ab	.02b	.03b	.01a
$F_4 - F_2$	.80**a	.78**a	.52**a	.84**a	.80**a	.54**b	.53**a	.55**a	.29**a

** significant at 1% level

* significant at 5% level

† coefficients for each type of intergeneration statistic within each cross followed by the same letter are not significantly different at the 5% level.

Analysis of variance for linoleic acid content in each cross was performed on the same set of F_2 single plants and their F_3 and F_4 progenies as for oleic acid. For the cross $P_1 \times P_2$, the F values for environments and families were highly significant (33.24 and 5.95 respectively) (Table 8). The heritability estimate for linoleic acid content was 50.3%. Environments and families were also a highly significant source of variation in $P_1 \times P_3$ with F values of 34.20 and 7.37 respectively. The heritability estimate was 56.1%. The F values for environments and families were highly significant but lower in magnitude for $P_2 \times P_3$ (27.85 and 2.31 respectively). The heritability estimate was also lower in magnitude (22.1%).

b) Means, ranges and standard deviations of F_2, F_3 and F_4 populations..

Cross $P_1 \times P_2$ had the lowest mean linoleic values in the F_2, F_3 and F_4 generations (17.9%, 15.5% and 19.1% respectively) (Table 9). All three generation means differed significantly from each other at the 5% level. For cross $P_1 \times P_3$, the F_3 mean (17.2%) was significantly lower than the F_2 mean (20.7%) and the F_4 mean (20.3%). The F_2 and F_4 means were not significantly different from each other. Cross $P_2 \times P_3$ had the highest mean linoleic values in the F_2, F_3 and F_4 generations (28.8%, 25.5% and 25.8% respectively). The F_2 mean was significantly higher than the F_3 and F_4 means. The F_3 and F_4 means were not significantly different from each other at the 5% level.

Table 8. Analysis of variance and heritability estimates for linoleic acid

Cross	Source	d.f.	Mean square	F value	Heritability Estimate
$P_1 \times P_2$	Families	50	27.69	5.95**	50.3%
	Environments	2	154.58	33.24**	
	Interaction	100	4.65		
$P_1 \times P_3$	Families	49	42.61	7.37**	56.1%
	Environments	2	197.65	34.20**	
	Interaction	98	5.78		
$P_2 \times P_3$	Families	49	10.36	2.31**	22.1%
	Environments	2	125.06	27.85**	
	Interaction	98	4.49		

** significant at 1% level

* significant at 5% level

Table 9. Frequency distributions for linoleic acid content of F_2 single plant selections and their F_3 and F_4 progenies.

Population	Class centers in percent of linoleic acid														n	Mean	Standard Deviation
	10	12	14	16	18	20	22	24	26	28	30	32	34	36			
$P_1 \times P_2$																	
F_2		5	5	3	4	3	0	5	2						27	17.9a*	4.8
F_3	1	8	5	3	3	5	2								27	15.5b	3.6
F_4			2	7	4	5	6	3							27	19.1c	3.1
$P_2 \times P_3$																	
F_2			10	2	2	2	1	2	4	3	2	0	0	1	29	20.7a	6.6
F_3	3	5	5	4	1	2	4	2	2	1					29	17.2b	5.4
F_4			4	3	6	3	4	5	3	0	1				29	20.3a	4.2
$P_2 \times P_3$																	
F_2								4	7	4	2	5	5		27	28.8a	3.6
F_3							4	6	8	4	2				25	25.5b	2.4
F_4						2	0	6	13	4	2				27	25.8b	2.3

* generation means within each cross followed by the same letter are not significantly different at the 5% level (L.S.D.).

For $P_1 \times P_2$, the ranges of linoleic acid content of F_2 single plants, F_3 families and F_4 families were 12.1% - 26.8%, 10.9% - 22.6% and 14.5% - 24.5%. For $P_1 \times P_3$, the ranges of the F_2, F_3 and F_4 were 13.2% - 36.0%, 9.9% - 28.9% and 14.0% - 30.3%. The ranges of the three generations in $P_2 \times P_3$ were 23.8% - 34.3%, 21.3% - 30.9% and 19.9% - 29.2%.

Cross $P_1 \times P_3$ had the greatest variation for percent linoleic acid content of the three crosses in each generation with standard deviations of 6.6, 5.4 and 4.2 in the F_2, F_3 and F_4 respectively. Cross $P_1 \times P_2$ had standard deviations of 4.8, 3.6 and 3.1 in the three generations. Cross $P_2 \times P_3$ had standard deviations of 3.6, 2.4 and 2.3.

c) Means of selected F_2 classes and their F_3 and F_4 progenies.

For $P_1 \times P_2$, the selected low, intermediate and high F_2 class means for linoleic acid content were significantly different from each other at the 5% level (13.1%, 18.1% and 24.6% respectively) (Table 10). The F_3 progeny means of F_2 classes (12.4%, 15.9% and 19.6%) were significantly different from each other as were the corresponding F_4 progeny means (16.3%, 19.7% and 22.0%).

For $P_1 \times P_3$, the selected low, intermediate and high F_2 class means were significantly different from each other (13.9%, 20.3% and 28.8% respectively). The corresponding F_3 progeny means were also significantly different from each other (12.5%, 16.0% and 23.6%). In the F_4, the progeny mean of the F_2 intermediate class (21.2%) was

Table 10. Means of linoleic acid content for selected low, intermediate and high F_2 classes and their F_3 and F_4 progenies.

Cross	Class	Generation		
		F_2	F_3	F_4
$P_1 \times P_2$	Low	13.1 a* (n=10)	12.4 a (n=10)	16.3 a (n=10)
	Intermediate	18.1 b (n=10)	15.9 b (n=10)	19.7 b (n=10)
	High	24.6 c (n=7)	19.6 c (n=7)	22.0 c (n=7)
$P_1 \times P_3$	Low	13.9 a (n=10)	12.5 a (n=10)	16.2 a (n=10)
	Intermediate	20.3 b (n=10)	16.0 b (n=10)	21.2 b (n=10)
	High	28.8 c (n=9)	23.6 c (n=9)	23.8 b (n=9)
$P_2 \times P_3$	Low	24.9 a (n=9)	24.5 a (n=8)	24.0 a (n=9)
	Intermediate	28.5 b (n=9)	25.6 a (n=8)	26.4 b (n=9)
	High	33.1 c (n=9)	26.4 a (n=9)	27.1 b (n=9)

* class means within each generation within each cross followed by the same letter are not significantly different at the 5% level (L.S.D.)

significantly higher than the F_4 progeny mean of the F_2 low class (16.2%) but was not significantly lower than the F_4 progeny mean of the F_2 high class (23.8%).

For $P_2 \times P_3$, the selected low, intermediate and high F_2 class means were significantly different from each other (24.9%, 28.5% and 33.1% respectively). However, no significant differences existed among the corresponding F_3 progeny means of the F_2 classes (24.5%, 25.6% and 26.4%). In the F_4, the progeny mean of the F_2 intermediate class (26.4%) was significantly higher than the progeny mean of the F_2 low class (24.0%) but was not significantly lower than the progeny mean of the F_2 high class (27.1%).

d) Average coefficients of variation for selected F_4 families.

Average coefficients of variation (A.C.V.) were calculated from ten F_4 single plant values for three sets of families including the three highest linoleic acid content F_4 families, the three lowest linoleic F_4 families and three intermediate linoleic content families. For each cross, the A.C.V. values for the three lowest and the three highest F_4 families were less than the A.C.V. value for the three intermediate families (Table 11). The A.C.V. values for the three classes were: 10.7%, 19.0% and 7.3% for $P_1 \times P_2$; 11.2%, 20.2% and 7.1% for $P_1 \times P_3$; 9.2%, 18.6% and 9.2% for $P_2 \times P_3$.

Table 11. Average coefficients of variation for linoleic acid content for three families in each of the low, intermediate and high classes of the F_4 generation.

Cross	Class		
	Low	Intermediate	High
$P_1 \times P_2$	10.7% (n=24)	19.0% (n=24)	7.3% (n=22)
$P_1 \times P_3$	11.2% (n=22)	20.2% (n=19)	7.1% (n=25)
$P_2 \times P_3$	9.2% (n=19)	18.6% (n=23)	9.2% (n=29)

Linolenic Acid

a) Intergeneration statistics

The intergeneration simple correlation, Spearman's rank correlation and regression values for linolenic acid content were considerably lower in magnitude than the corresponding set of values for oleic and linoleic acids. There were no significant differences among the values for each type of intergeneration statistic within each cross.

For the cross $P_1 \times P_2$, the intergeneration simple correlation coefficients for F_3 versus F_2, F_4 versus F_3 and F_4 versus F_2 were .20, .39 and .29 respectively (Table 12). The corresponding Spearman's rank correlation coefficients were .21, .40 and .25. For both of these intergeneration statistics, only the F_4 versus F_3 values were significant. The regression values for $P_1 \times P_2$ were .12, .68 and .30. None of these values was significant.

For $P_1 \times P_3$, the three intergeneration simple correlation values were .01, -.02 and .22. The corresponding Spearman's rank values were .04, .04 and .24. The three regression values were .01, -.02 and .21. None of these values was significant.

For $P_2 \times P_3$, the intergeneration simple correlation values were -.21, .13 and -.29. The corresponding Spearman's rank values were -.23, .04 -.23. The three regression values were -.16, .22 and -.36. None of these values was significant.

Table 12. Intergeneration simple correlation, Spearman rank correlation and regression coefficients for linolenic acid values among F_2 single plant selections and their F_3 and F_4 progenies.

Generations	Cross								
	$P_1 \times P_2$ (n=26)			$P_1 \times P_3$ (n=29)			$P_2 \times P_3$ (n=25)		
	r	rank r	b	r	rank r	b	r	rank r	b
$F_3 - F_2$	.20a†	.21a	.12a	.01a	.04a	.01a	-.21a	-.23a	-.16a
$F_4 - F_3$	.39*a	.40*a	.68a	-.02a	.04a	-.02a	.13a	.04a	.22a
$F_4 - F_2$	.29a	.25a	.30a	.22a	.24a	.21a	-.29a	-.23a	-.36a

** significant at 1% level

* significant at 5% level

† coefficients for each type of intergeneration statistic within each cross followed by the same letter are not significantly different at the 5% level.

Table 13. Analysis of variance and heritability estimates for linolenic acid

Cross	Source	d.f.	Mean square	F value	Heritability Estimate
$P_1 \times P_2$	Families	50	4.64	1.59*	10.0%
	Environments	2	114.96	39.37**	
	Interaction	100	2.92		
$P_1 \times P_3$	Families	49	4.78	1.40	6.7%
	Environments	2	150.80	44.22**	
	Interaction	98	3.41		
$P_2 \times P_3$	Families	49	3.49	1.05	0.20%
	Environments	2	85.85	25.78**	
	Interaction	98	3.33		

** significant at 1% level

* significant at 5% level

The analysis of variance for linolenic acid content in the cross $P_1 \times P_2$ indicated that the F value for environments was highly significant (39.37) (Table 13). The F value for families was significant at the 5% level (1.59). The heritability estimate for linolenic acid content was only 10.0%. For cross $P_1 \times P_3$, the F value for environments was highly significant (44.22). The F value for families (1.40) was not significant. The heritability estimate was 6.7%. Environments (F = 25.78) were a highly significant source of variation in $P_2 \times P_3$. Families (F = 1.05) were not a significant source of variation. The heritability estimate was .20%.

b) Means, ranges and standard deviations of F_2, F_3 and F_4 populations.

For $P_1 \times P_2$, the F_3 mean (8.7%) was significantly lower than the F_2 mean (11.2%) and the F_4 mean (11.3%) at the 5% level (Table 14). The F_2 and F_4 means were not significantly different from each other. For $P_1 \times P_3$, the F_2, F_3 and F_4 means were 12.5%, 9.4% and 11.4% respectively. All three generation means differed significantly from each other. For $P_2 \times P_3$, the F_3 mean (9.3%) was significantly lower than the F_2 mean (11.9%) and the F_4 mean (12.0%). The F_2 and F_4 means were not significantly different from each other at the 5% level.

Table 14. Frequency distributions for linolenic acid content of F_2 single plant selections and their F_3 and F_4 progenies.

	Class centers in percent of linolenic acid									
Population	6	8	10	12	14	16	18	n	Mean	Standard Deviation
$P_1 \times P_2$										
F_2		7	5	10	5	0	1	28	11.2a*	2.3
F_3	3	13	9	1				26	8.7b	1.4
F_4	1	4	7	8	8			28	11.3a	2.4
$P_1 \times P_3$										
F_2		1	9	5	11	3		29	12.5a	2.2
F_3	3	8	12	6				29	9.4b	1.7
F_4		2	12	9	4	2		29	11.4c	2.1
$P_2 \times P_3$										
F_2			11	7	7	2		27	11.9a	1.9
F_3	2	8	12	3				25	9.3b	1.5
F_4		5	6	6	6	4		27	12.0a	2.5

* generation means within each cross followed by the same letter are not significantly different at the 5% level (L.S.D.).

For $P_1 \times P_2$, the ranges of the F_2 single plants, F_3 families and F_4 families were 7.8% - 17.5%, 5.8% - 11.7% and 6.4% - 14.4%. The ranges of the three generations in $P_1 \times P_3$ were 8.4% - 15.7%, 5.8% - 12.8% and 8.7% - 15.8%. For $P_2 \times P_3$, the ranges in the three generations were 9.0% - 15.6%, 6.6% - 12.3% and 8.2% - 15.7%.

Cross $P_1 \times P_2$ had standard deviations of 2.3, 1.4 and 2.4 in the three generations. Cross $P_1 \times P_3$ had standard deviations of 2.2, 1.7 and 2.1 in the three generations. For $P_2 \times P_3$, the standard deviations in the three generations were 1.9, 1.5 and 2.5.

c) Means of selected F_2 classes and their F_3 and F_4 progenies.

For $P_1 \times P_2$, the selected low, intermediate and high F_2 class means for linolenic acid content were significantly different from each other at the 5% level (8.7%, 11.4% and 13.7% respectively) (Table 15). No significant differences existed among the corresponding means of F_3 progeny of F_2 classes (8.2%, 9.1% and 8.8%). In $P_1 \times P_2$, the F_4 progeny mean of the F_2 intermediate class (12.4%) was significantly higher than the means of F_4 progenies of the F_2 low and high classes (10.2% and 11.2% respectively). The F_4 progeny means of the F_2 low and high classes were not significantly different from each other.

Table 15. Means of linolenic acid content for selected low, intermediate and high F_2 classes and their F_3 and F_4 progenies.

Cross	Class	Generation		
		F_2	F_3	F_4
$P_1 \times P_2$	Low	8.7 a* (n=10)	8.2 a (n=8)	10.2 a (n=10)
	Intermediate	11.4 b (n=9)	9.1 a (n=9)	12.4 b (n=9)
	High	13.7 c (n=9)	8.8 a (n=9)	11.2 a (n=9)
$P_1 \times P_3$	Low	9.8 a (n=9)	9.6 a (n=9)	11.1 a (n=9)
	Intermediate	12.6 b (n=10)	8.8 a (n=10)	11.1 a (n=10)
	High	14.9 c (n=10)	9.9 a (n=10)	12.0 a (n=10)
$P_2 \times P_3$	Low	9.9 a (n=9)	10.0 a (n=9)	13.0 a (n=9)
	Intermediate	11.8 b (n=10)	8.8 a (n=9)	11.2 b (n=10)
	High	14.2 c (n=8)	9.2 a (n=7)	11.9 b (n=8)

* class means within each generation within each cross followed by the same letter are not significantly different at the 5% level (L.S.D.).

The selected low, intermediate and high F_2 class means for $P_1 \times P_3$ differed significantly from each other (9.8%, 12.6% and 14.9% respectively). But, no significant differences were observed among the corresponding F_3 progeny means (9.6%, 8.8% and 9.9%). Similarly, no significant differences were observed among the corresponding F_4 progeny means (11.1%, 11.1% and 12.0%).

For $P_2 \times P_3$, selected low, intermediate and high F_2 class means were all significantly different from each other (9.9%, 11.8% and 14.2% respectively). There were no significant differences among the corresponding F_3 progeny means (10.0%, 8.8% and 9.2%). The mean of F_4 progeny of the F_2 intermediate class (11.2%) was significantly lower than the mean of progeny of the F_2 low class (13.0%) but was not significantly lower than the mean of progeny of the F_2 high class (11.9%).

d) Average coefficients of variation for selected F_4 families.

Average coefficients of variation (A.C.V.) were calculated for the three sets of families including the three highest linolenic acid content families, the three lowest linolenic families and three intermediate linolenic families. For each cross the A.C.V. values for the three low and the three high F_4 families

Table 16. Average coefficients of variation for linolenic acid content for three families in each of the low, intermediate and high classes of the F_4 generation.

Cross	Class		
	Low	Intermediate	High
$P_1 \times P_2$	16.4% (n=17)	11.3% (n=26)	15.0% (n=22)
$P_1 \times P_3$	13.6% (n=17)	10.1% (n=24)	10.9% (n=20)
$P_2 \times P_3$	20.0% (n=23)	13.7% (n=19)	15.6% (n=20)

were lower than the A.C.V. value for the three intermediate families (Table 16). The A.C.V. values for the three low, intermediate and high families were: 16.4%, 11.3% and 15.0% for $P_1 \times P_2$; 13.6%, 10.1% and 10.9% for $P_1 \times P_3$; 20.0%, 13.7% and 15.6% for $P_2 \times P_3$. Thus, the A.C.V. values indicated greater variation for the three low and high families than for the three intermediate families.

Correlation Coefficients

Highly significant negative correlations were observed between oleic and linoleic acids in segregating generations (Table 17). The F_2 correlation coefficients between oleic and linoleic acids in $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_3$ (-.92, -.96 and -.92 respectively) were very similar in magnitude to the corresponding F_3 correlations (-.96, -.84 and -.88). The corresponding F_4 correlations (-.82, -.89 and -.71) were similar in magnitude to those of the F_2 and F_3 generations with the exception of the correlation for $P_2 \times P_3$.

The correlation coefficients between oleic and linolenic acids were highly significant and negative in value. They were lower in magnitude than the correlations between oleic and linoleic. The three F_2 correlation coefficients (-.63, -.58 and -.71) were similar in magnitude to the corresponding F_3 correlations (-.67, -.64 and -.71). The corresponding F_4 correlations (-.49, -.39 and -.64) were lower in magnitude than those of the F_2 and F_3 generations.

Table 17. Simple correlation coefficients for pairs of fatty acids for parental, F_1, F_2, F_3 and F_4 populations.

Population	Oleic and Linoleic	Oleic and Linolenic	Linoleic and Linolenic
P_1	-.77**	-.60**	.30
F_2 (1 x 2)	-.92**	-.63**	.43**
F_3 (1 x 2)	-.96**	-.67**	.46**
F_4 (1 x 2)	-.82**	-.49**	.12*
P_2	-.83**	-.57**	.40*
P_1	-.77**	-.60**	.30
F_2 (1 x 3)	-.96**	-.58**	.40**
F_3 (1 x 3)	-.84**	-.64**	.64**
F_4 (1 x 3)	-.89**	-.39**	.11
P_3	-.35	-.61**	-.31
P_2	-.83**	-.57**	.40*
F_2 (2 x 3)	-.92**	-.71**	.52**
F_3 (2 x 3)	-.88**	-.71**	.34*
F_4 (2 x 3)	-.71**	-.64**	.29*
P_3	-.35	-.61**	-.31

** significant at 1% level
* significant at 5% level

Correlations between linoleic and linolenic in segregating generations were all positive and were more variable in magnitude compared with the above correlations. The F_2 correlations in $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_3$ (.43, .40 and .52 respectively) agreed reasonably well with the corresponding F_3 correlations (.46, .64 and .34). However, the corresponding F_4 correlations (.12, .11 and .29) were considerably lower in magnitude.

Linolenic - Linoleic Ratio

The ability of the 18:3/18:2 ratio to identify low linolenic acid content families were variable. For F_3 families in cross $P_1 \times P_2$, six of the ten lowest 18:3/18:2 ratios corresponded to the low linolenic acid category (Table 18). For $P_1 \times P_3$, only four of the ten lowest ratios could be matched with the low linolenic category. However, for $P_2 \times P_3$, nine of the ten lowest 18:3/13:2 ratios fell into the low linolenic acid category.

For F_4 families, in cross $P_1 \times P_2$, five of the ten lowest 18:3/18:2 ratios corresponded to families in the low linolenic acid category. For $P_1 \times P_3$, only three of the ten lowest ratios corresponded to families in the low linolenic category. For $P_2 \times P_3$, six of the ten lowest ratios fell into the low linolenic acid category.

Table 18. Classification of ten lowest 18:3/18:2 families on the basis of linolenic acid content.

	F_3 Generation		F_4 Generation	
Cross	Low Linolenic	High Linolenic	Low Linolenic	High Linolenic
$P_1 \times P_2$	6	4	5	5
$P_1 \times P_3$	4	6	3	7
$P_2 \times P_3$	9	1	6	4

DISCUSSION

The main purpose of this study has been to examine the degree of success expected in recovering a desirable C-18 fatty acid composition in early generations derived from crosses among low erucic lines of Brassica napus. Selection would be directed at increasing linoleic acid and reducing linolenic acid content. If the desirable combination can be recovered, then such a C-18 fatty acid package could be transferred to a low glucosinolate, low erucic acid variety.

In cross $P_1 \times P_2$, the average values of the three intergeneration simple correlation and Spearman's rank correlation coefficients for percent oleic acid were .70 and .69 respectively. The corresponding average values for $P_1 \times P_3$ were .78 and .70 and for $P_2 \times P_3$ they were .18 and .16. The high intergeneration correlations for the first two crosses demonstrated that the oleic acid content of the F_2 single plants was a good indicator of the F_3 and F_4 progeny values and F_3 values were a good indicator of F_4 progeny values. Broad sense heritabilities estimated by regression coefficients and analysis of variance were lower in magnitude but indicated the same trend among crosses as the corresponding correlation coefficients. The average regression heritabilities for percent oleic acid in crosses $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_3$ were .60, .61 and .13 respectively. The corresponding heritability estimates from the analysis of variance (31.8%, 39.0% and 14.6%) were considerably lower in magnitude than those reported by

Thomas (1974) for the same three crosses (75%, 77% and 58%). Regression heritabilities in this study are similar in magnitude to heritability estimates for percent oleic acid in field grown flax (65%) but are higher in magnitude than heritability estimates for flax grown under a controlled environment (9.0%) (Comstock et al 1960).

The average values of the three intergeneration simple correlation and Spearman's rank correlation coefficients for percent linoleic acid in cross $P_1 \times P_2$ were .79 and .76 respectively. The corresponding average values for $P_1 \times P_3$ were .85 and .82, while for $P_2 \times P_3$ they were .18 and .16. The high intergeneration correlations for the first two crosses demonstrated that the linoleic acid content of the F_2 single plants was a good indicator of the F_3 and F_4 progeny values and F_3 values were a good indicator of F_4 progeny values. These intergeneration values were higher than those observed for soybeans where F_3 versus F_2 simple correlations for percent linoleic in five crosses ranged from -.090 to .495 (White et al 1961). The regression heritabilities and heritabilities based on the analysis of variance for linoleic acid were lower in magnitude but indicated the same trend among crosses as the corresponding correlation coefficients. The average regression heritabilities in crosses $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_3$ were .59, .64 and .18 respectively. The differences between the regression heritabilities and the heritabilities from the analysis of variance were not as great for linoleic as for oleic acid. The heritabilities from the analysis of variance in each cross were 50.3%, 56.1% and 22.1%. Heritability estimates for linoleic acid in this study were

lower in magnitude than those reported by Thomas (1974) for the same three crosses (81%, 81% and 43%). The heritabilities in this study are higher than those for flax where percent linoleic acid was reported to be virtually non-heritable (Comstock et al 1960).

The average values of the three intergeneration simple correlation and Spearman's rank correlation coefficients for percent linolenic acid in $P_1 \times P_2$ were .29 and .29 respectively. The corresponding average values for $P_1 \times P_3$ were .07 and .11, while for $P_2 \times P_3$ they were -.12 and -.14. These very low intergeneration values demonstrated that the linolenic acid content of the single F_2 plants was a poor indicator of the F_3 and F_4 progeny values and F_3 values were a poor indicator of F_4 progeny values. These values were lower than those observed for soybeans where F_3 versus F_2 simple correlations for percent linolenic acid ranged from .055 to .635 (White et al 1961). The average regression heritabilities for percent linolenic acid in $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_3$ were .37, .07 and -.10 respectively. The heritabilities from the analysis of variance in the three crosses were 10.0%, 6.7% and 1.0%. Heritability estimates for linolenic acid in this study are lower in magnitude than those reported by Thomas (1974) for the same three crosses (54%, 41% and 30%). Heritabilities in this study are lower in magnitude than heritabilities for percent linolenic acid in field grown flax (72.9%) but are similar to heritabilities for flax grown under a controlled environment (1.1%) (Comstock et al 1960).

The heritabilities for oleic and linoleic acids are higher than the heritabilities for linolenic acid. This supports the results obtained by Thomas (1974). For oleic and linoleic acids in this study, the two methods of estimating heritability produced higher values in $P_1 \times P_2$ and $P_1 \times P_3$ than in $P_2 \times P_3$. These results are also similar to those reported by Thomas (1974) for the same three crosses.

For oleic acid content in crosses $P_1 \times P_2$ and $P_1 \times P_3$, the low and high F_2 classes were significantly different. This was also true of the F_3 and F_4 progeny means of these classes. In cross $P_2 \times P_3$ significant differences were found only at the F_2 level. For linoleic acid content, the low and high F_2 classes and their F_3 and F_4 progeny means were significantly different in all cases except for the F_3 progeny of $P_2 \times P_3$. The low and high F_2 classes, based on linolenic acid content, were significantly different. However, no significant differences were observed in the F_3 and only the F_4 progeny of the low and high F_2 classes of $P_2 \times P_3$ were significantly different. These results would be expected on the basis of the intergeneration correlation coefficients and heritability estimates.

Comparison of F_2, F_3 and F_4 generation means indicated higher levels of oleic and lower levels of linoleic and linolenic acids in greenhouse grown material than in field grown material for all three crosses. These results are in agreement with those of Bechyne and Kondra (1970). Similar trends were observed for oleic

and linolenic acids in flax (Comstock et al 1960).

The average coefficients of variation (A.C.V.) indicate the degree of homogeneity in each of the three sets of F_4 families including the three highest, the three lowest and three intermediate families for each fatty acid in each cross. For oleic acid, the A.C.V. values in the low and high sets of families were lower than the A.C.V. values for the intermediate sets of families in $P_1 \times P_2$ and $P_1 \times P_3$. The same trend existed for linoleic acid in all three crosses. The situation was reversed for linolenic acid in all three crosses where the A.C.V. values for the low and high sets of families were greater than the A.C.V. values for the intermediate sets of families. This implies that the original F_2 partition of single plants into the low, intermediate and high classes on the basis of oleic and linoleic acid content was successful in assigning a greater number of homozygous plants to the low and high classes.

Correlation coefficients indicated that oleic was highly negatively correlated with linoleic (-.96 to -.71) and linolenic (-.71 to -.39), while linoleic and linolenic were positively correlated (.11 to .64). These are in general agreement with results reported by Thomas (1974) and Stefansson and Storgaard (1969). Thomas (1974) indicated that the relative ratios of oleic and linoleic acids are probably under the control of one genetic system. This would explain the consistently high negative correlations between oleic and linoleic acids. Thomas (1974) indicated that the ratio of

linoleic and linolenic is not controlled by the same genetic system. Hence, it is not unexpected that in the present study the F_4 correlations between linoleic and linolenic are considerably lower in magnitude than the corresponding F_2 and F_3 correlations. The non-significant positive F_4 correlations between linoleic and linolenic suggest the probability of effective selection for high levels of linoleic acid independent of linolenic acid content.

The 18:3/18:2 ratio is determined by a spectrophotometric technique which does not provide actual values of linolenic acid content. Therefore, the ability of the 18:3/18:2 ratio to identify families having low linolenic levels depends on a highly negative correlation between linoleic and linolenic acids. The correlations between linoleic and linolenic acids varied from -.31 in P_3 to .64 in the F_3 of $P_1 \times P_3$. In their work, Rakow and McGregor (1973) considered a range of .22 to .35 for ratio values to be low in linolenic acid. The cross having the largest number of desirable ratios was the F_3 of $P_2 \times P_3$ where nine of ten low ratio values corresponded with families having low linolenic levels. However, the 18:3/18:2 ratio was less successful in identifying low linolenic families in all other crosses in the F_3 and F_4 generations. The effectiveness of the 18:3/18:2 ratio in identifying low linolenic acid types was not as great as expected.

Parent 3 has a desirable high level of linoleic acid. Parent 1 has a fatty acid composition similar to existing low erucic, low glucosinolate germplasm. Since high levels of linoleic

acid were recovered in segregating generations, it is probable that the high linoleic character could be transferred to existing low erucic, low glucosinolate germplasm.

The reduction of linolenic content appears to be more difficult due to lack of genetic variability and low heritability. Linolenic acid could be reduced by various methods. Rakow and McGregor (1973) utilized induced mutations in B. napus and made selections based on the 18:3/18:2 ratio. However, they found that low linolenic mutants reverted back to higher levels when grown in different environments. A problem with induced mutations in B. napus is the fact that it is an amphidiploid (B. campestris and B. oleracea) and the expression of a successful mutation in one genome could be buffered by the second genome. A second possibility would be to transfer low levels of linolenic acid from wild or cultivated species of Brassica to cultivars of B. napus by means of interspecific crosses. However, most wild Brassica species have significant amounts of erucic acid. A third possibility would involve the application of mutation and selection in B. campestris and B. oleracea with the purpose of reducing the linolenic content in each species. The artificial amphidiploid form of B. napus could then be produced and would likely have seed low in linolenic acid. The artificial amphidiploid could then be used in any regular breeding program. However, this method would be extremely time consuming since it would require screening large populations and the probability of obtaining the desired mutation in both species would be low.

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